

STUDIES ON PHYSICAL PROPERTIES OF NATIVE, GELATINIZED AND ACETYLATED RICE STARCHES

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ABSTRACT

The physico-chemical properties of native, acetylated and gelatinized starches from commercial rice exhibited that, the gelatinized starch showed higher amylose content in comparison to acetylated one which showed no significant increasing in amylose compared with native rice starch. The swelling power in acetylated and gelatinized starches are high compared to unmodified starch. The gelatinized starch (GS) showed the highest amylase content, followed by acetylated one, which have not a significant difference in comparison to native rice starch. Its well known that, native starch have a poor solubility, however, acetylated one showed a higher solubility, and the gelatinized is the superior compared to them. The scanning electron spectroscopy did not show any significant difference between the external morphology of native and acetylated starches but in GS the starch granules size increased greatly. onset temperature (T_o) and peak temperature (T_p) increased for acetylated starch, while conclusion temperature (T_c) decreased but gelatinized starch recorded a decreasing in onset temperature (T_o) and increasing in T_p , T_c . enthalpies of gelatinization ΔH_{gel} values increased for all modified starches in comparison to native starch.

Keywords: Rice starch; Acetylation; Physico-chemical; Morphological; Thermal; Retrogradation

INTRODUCTION

Starch, a polysaccharide, is a major food ingredient providing energy often at low cost in the human diet. Starch could be modified to resistant types for the enzyme in the digestive system or irritant ones. Resistant starches (RS) have many applications in both food and industrial products. Due to the low shear stress, thermal decomposition, high retrogradation and syneresis of the native starch, its uses became limited. (Betancur *et al.*, 1997). Resistant starch can be added to starchy foods to improve nutritional value. Numerous chemical modifications may be applied to starch to improve its properties that are useful for particular applications. One of the common starch modification is acetylation, which is the esterification of starch polymers with acetyl groups to form starch acetates (Jarowenko, 1986). Another modification technique is autoclave treatment (pre-gelatinization) which is the retrogradation of starch polymers to form retrograded pre-gelatinized starches (Berry 1986).

Acetylation of starches has been applied to impart the thickening needed in food applications. Acetylated starches have improved properties over their native counterparts and have been used for their stability and resistance to retrogradation (Wurzberg, 1987).

The extent changes in of physico-chemical properties for the acetylated starch compared to the native starch is proportional to the degree of acetylation or degree of substitution incorporated into the starch molecules (Phillips *et al.*, 1999).

Heat-moisture treated and retrograded high-amylose starches are attractive sources of dietary fiber because they are white in color, bland in flavor, smooth in texture and carry no charge (Seib and Woo 1999). Water sorption isotherms of both kinds of RS and swelling power and solubility are important properties to be considered when RS is applied to new products.

Modified starches are extensively used in a large variety of foods including baked goods, canned pie fillings, sauces, retorted soups, frozen foods, baby foods, salad dressings, and snack foods (Wurzburg, 1995). The availability of corn to the starch industry is decreasing, day by day, because of increased demand by industries involved in the production of breakfast cereals and snacks. The broken rice, which is cheaper than corn and is available in high ratio, can be used in the production and modification of starch. The present study aimed to produce and evaluate the physical properties of autoclaved-frozen and acetylated starches which modified from the starch isolated from commercial brooked rice kernels.

MATERIALS AND METHODS

1. Materials

Commercial bricked rice kernels from El-Sharkia Bleaching Rice Co. in Zagazig City-Egypt.

2. Methods

2.1. Starch isolation

Starch was isolated from various rice cultivars by alkali extraction of the protein, explained by (Nikuni & Hizukuri, 1958).

2.2. Preparation of Resistant Starch (GS) :

It was applied according to De-Deckere *et al.* (1993). One kilogram of starch was fully gelatinized with 3 liter of tap water in autoclave at 121°C and 1 bar pressure for 60 min. The mixture was cooled to room temperature and then stored at -20°C for 24 hr. After thawing the starch, dried in oven air drier at 50°C over night and then grinded.

2.3. Acetylation of isolated rice starches:

Acetylation of rice starches carried out according to method described by Phillips *et al.* (1999).

2.4. Acetyl percentage and degree of substitution.

The Acetyl percentage and degree of substitution of (DS) were determined titrimetrically according to Wurzburg (1978) and (Whistler & Daniel, 1995).

The acetylation percent calculated from the following equation.

$$\text{Acetyl \%} = \frac{(\text{Blank} - \text{Sample}) \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{Sample weight}}$$
$$\text{DS} = \frac{162 \times \text{Acetyl \%}}{4300 - (42 \times \text{Acetyl \%})}$$

D.S. = degree of substitution

162 = M.W. of anhydroglucose unit.

43 = Molecular weight of acetyl group. CH₃CO-

2.5. Swilling Power And Solubility.

The swelling power of starches determined as described by Subramanian *et al.* (1994).

Solubility was determined as the ratio between weighted of soluble starch and weight of sample.

2.6. Scanning Electron Spectroscopy.

Starch samples were scanned by electron Spectroscopy according to A.O.A.C. (2000)

2.7. X-Ray Diffraction.

Samples ($\approx 0.7g$) were pressed into 10×25 mm pellets on a hydraulic press (2.300 kg). scanned an angular range (2θ) between 2θ and 60θ at a scan rate of 0.5θ /min. as described by A.O.A.C. (2000).

2.8. Thermal Properties of Starches Determined by DSC.

Thermal properties of starches were analyzed by differential scanning calorimeter (DSC-7, Perkin Elmer Corp., Norwalk, CT) equipped with an intra cooler II system and pyres thermal analysis software (Perkin-Elmer). The samples were heated at $10^{\circ}C/min.$ over a temperature range of $25-250^{\circ}C$. The gelatinization temperature and enthalpy change ΔH were determined following the procedure of Kasemsuwan *et al.* (1995). Enthalpy change (ΔH), onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) were computed.

RESULTS AND DISCUSSION

1- Amylose content.

The iodine reaction gives a bluish colour with native starch and can be used under standard conditions to determine the amylose fraction. In this respect the main components of starch (amylose and amylopectin) were determined before and after modifications. Its clear in table (1) that the amylose content of native rice starch, RS3 and starch acetate. The results indicated pronounce changes in the content of amylose and amylopectin under the conditions of preparing starch derivatives. Amylose content of rice starch exhibited significant decreasing after acetylation. The presence of acetyl groups has been reported to interfere with the functioning of amylose and amylopectin fractions of starch and it affects on the absorption of iodine during amylose testing (Betancur *et al.*, 1997). The acetyl groups introduced in rice starch chains impeded the formation of the helical structure of amylose in some areas, by sterical hindrance and in consequence, formation of amylose-iodine complex, which resulted in underestimation of (Gonzalez and Perez 2002).

Table(1): Contents of amylose and amylopectein for native rice starch and its derivatives.

Starch Substrate.	Amylose.	Amylopectein by Deference
Native starch.	20.4	79.6
Starch acetate.	16.62	83.38
Gelatinized Starch.	21.2	78.8

*On dry basis

2- Swilling Power and Solubility.

The swilling power and solubility of all samples were determined at (25°C and 95°C) and the results presented in table (2), the results showed that the swelling power values for these samples were increased as swelling temperature.

At 25°C native starch and starch acetate exhibited negligible variation but in gelatinized starch showed great increasing in swelling power than it in native starch this could be attributed to the modification occurred the starch structure as degraded some bonds in starch polymer fractions (amylose and amylopectin) at autoclaving and icing treatments during gelatinized starch was prepared. But in starch acetate the cross-linkage were formed may be occurred strong structure of starch granule caused decrease in swelling power for starch derivatives samples than control.

Table (2): Swelling power, solubility, % acetyl and degree of substitution for native rice starch and its derivatives.

Starch substrate	Swelling power		Solubility		% Acetyl	D.S
	25°C	95°C	25°C	95°C		
Native starch	1.48	16.61	0.82	18.01	-	-
Starch acetate.	1.51	17.70	1.02	19.21	4.02	0.158
Gelatinized Starch.	3.98	20.85	3.56	26.45	-	-

D.S = degree of substitution.

At 95°C the swelling power for native starch and its derivatives were also varied and showed different water absorption capacity and consequently different swelling power values.

The increase in swelling power values could be attributed to the repulsion between negatively charged groups on starch molecules and to relaxation of bonding forces within starch granules with an increase in temperature (Lim and Sieb 1993) Reported that the swilling power of starch was depending upon the degree of hydrolysis and number of introduced ionic groups.

On the other hand, the same results in table (2) also showed that the native starch and its derivatives had different percentage of solubility that increased at 25oC to 95oC. solubility percentage for all these samples were increased as the temperature of treatment increased. The low solubility values for native starch could be attributed to increase of bonding forces within starch molecules and resistance towards solubility. Under the same conditions there is an increase in solubility for starch acetate with an increase in temperature. This increase in solubility it may be due to (-OH, CH₃CO=) group or residual activity in starch (Aiyeye et al., 1993).

The introduction of acetyl groups in starch could have also facilitated the access of water to amorphous areas. The acetylated starches with higher acetyl content showed higher solubilities. The acetylation of starches with low amylose content showed limited change in solubility. Similar trends of increase in swelling power and solubility upon acetylation has been reported earlier for potato and corn starches (Singh et al., 2004).

GS showed swelling power at 95°C higher than it at 25°C and the same for solubility it may be due to debranched fractions were produced during heat-moisture treatment of starch (Shi and Trzasko 1997). And the melting in the crystalline region. Even though the granules of gelatinized starch were swollen, the soluble carbohydrate that was leached was only 0.5% because the starch molecules had been linked together. The solubility of GS wheat starch at 95°C was much higher than cross-linked starches because GS wheat starch contained some amorphous material that contributed to swelling and solubility Shin *et al.* (2003).

3- Degree of substitution of acetyl groups.

The acetyl % and DS were observed to be lower in this study as reported earlier for potato and corn starches treated with acetic anhydride under similar conditions. This may be attributed to difference in amylose content and granular structure of rice, potato and corn starches. The variation in degree of substitution among different starches may be also due to difference in intragranule packing of rice starches. Because the way in which the amylose chain are packed in amorphous regions as well as arrangement of amylose and amylopectin chains could affect the chemical substitution reaction in the glucose units of starch macromolecules Islam *et al.* (1974).

4- Morphological properties.

The scanning electron spectrographs in fig (1-2). Showed the presence of mainly native granules having size in the range of 4.9µm-7.8µm and the figure also showed the presence of hexagonal granules in addition to normally present pentagonal granules. The results were in agreement as those of Sodhi and Singh (2005). Scanning electron microscopy revealed decrease in acetylated rice starch granules the granules size ranged between 2.4µm-4.9µm. it may be due to the cross-linked were formed cause the starch polymers chains become nearest than there in native starch granules. But in GS the autoclaving and freezing-thawing treatment cussed fragments in molecule structure its cussed increasing in granules size.

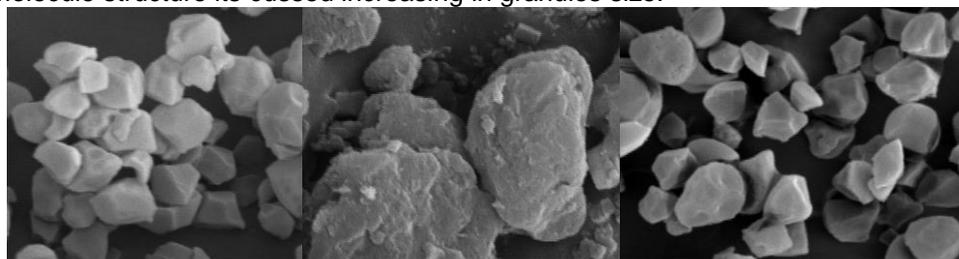


Fig (1): Native Starch as Scanning Electron Microscopy at Magnification 4000 Times.

Fig (2): Gelatinized starch as scanning electron microscopy at magnification 4000 times.

Fig (3): starch acetate as scanning electron microscopy at magnification 4000 times.

5- X-ray diffraction.

Native and acetylated Starches did not show any changes in the crystalline form as reported by Lim *et al.* (2004). Its exhibited peaks at $2\theta = 15^\circ, 17^\circ, 18^\circ,$ and 23° which were characteristic of A-type crystallinity

(Figures. 14-17), while the diffraction patterns of RS3 were typical of B-type crystallinity, as indicated by the pronounced peaks at $2\theta = 17^\circ$ and 20° (Keren *et al.*, 2003). Besides the B-type profile, RS3 exhibited an additional peak at $2\theta = 20^\circ$. A peak in this location is characteristic of V-type crystallinity. Similar combination of B- and V-type crystallinity in starch was previously reported by Sievert *et al.* (1991). The crystallinity of the residue was not significantly changed, but RS3 showed high intensity of the peak at $2\theta = 17^\circ$ compared to other peaks. As mentioned above, RS consists of crystallized and amorphous regions. Low crystallinity in RS3 may be due to the crystalline region of RS3 was affected by autoclave treatment, the RS3 showed reduction of the peak at $2\theta = 20^\circ$, probably due to the hydrolysis of the amylose–lipid complex formed during the heating process. as suggested by the previous study (Wang *et al.*, 2003),

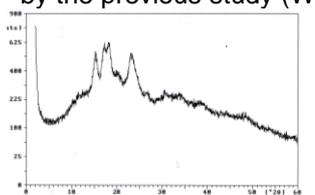


Fig. 4. X-ray diffractogram of native starch.

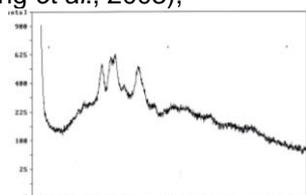


Fig. 5. X-ray diffractogram of starch acetate.

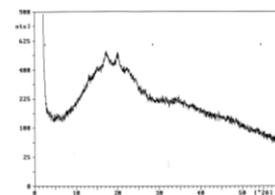


Fig. 6. X-ray diffractogram of gelatinized starch.

6- Thermal Properties of rice starches.

DSC results of starches separated from milled rice summarized in Table 3. Figures. (7-9) presents the gelatinization thermograms of starches separated from different rice cultivars. The transition temperatures (T_o , T_p and T_c), enthalpies of gelatinization (ΔH_{gel}), range (R) and enthalpies of retrogradation (ΔH_{ret}) of the rice starch differ significantly. T_p and T_c were higher than of those in native starch. But T_o lower than native starch. T_c and T_p did not differ significantly in native and gelatinized starches. Starch acetate showed the highest T_o and T_p and significant highest T_o . ΔH_{gel} was observed to be highest (3.4928 J/g) and (-38.1348 J/g) for gelatinized starch whereas starch acetate showed low ΔH_{gel} value (-29.222 J/g) (Cooke & Gidley 1992). The differences in T_o , T_p , T_c and ΔH_{gel} in starches from different rice patterns may be attributed to differences in degraded starch polymers (amylose and amylopectin) contents and granular structures. Larger T_o and T_p values for starch acetate may be attributed to the compact nature of small starch granules and higher degree of molecular order of the granules (Krueger *et al.*, 1987). Because amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting point of crystalline regions and the energy for starting gelatinization be higher (Flipse *et al.*, 1996). More energy is needed to initiate melting in the absence of amylose-rich amorphous regions (Krueger *et al.*, 1987). The differences in transition temperatures in rice starches may also be due to differences in lengths of the amylopectin (Jane *et al.*, 1999). The transition temperature and enthalpies observed for rice starches in the present study were found to fall within a range similar to those already reported Sodhi and Singh (2003). The endothermic peaks of starches, after storage of gelatinized rice starches at

4°C for 7 days appeared -38.1348 J/g of amylopectin branch chains has been reported to occur in a less ordered manner in stored starch gels than in native starches. This explains the occurrence of amylopectin retrogradation endotherms at a temperature range below that for gelatinization (Ward *et al.*, 1994). The value of ΔH_{ret} may be depending on the differences in amylose–amylopectin ratios and granular structures (Hizukuri 1996). The amylopectin and intermediate materials play a significant role in starch retrogradation during refrigerated storage. The intermediate materials with longer chains than amylopectin may also form longer double helices during reassociation under refrigerated storage conditions (Yamin *et al.*, 1999).

This difference in range of gelatinization suggests that the degrees of heterogeneity of crystallites within granules of the studied starches are different. Furthermore, high amylose starches with longer average chain were reported to exhibit higher transition temperatures than others low average chain (Jane *et al.*, 1999). The differences in gelatinization temperatures among rice starches may be attributed to the contribution of the following factors: starch composition (amylose to amylopectin ratio), granular architecture (crystalline to amorphous ratio), and molecular structure of amylopectin (extent of branching, unit chain length, and polydispersity) (Gunaratne and Hoover 2002). For individual native rice starches, the enthalpies of gelatinization starch had the highest heat of gelatinization because, being exclusively composed of only amylopectin, its starch granule's crystallinity is considered higher than that of starch acetate.

Kreuger *et al.* (1987) reported that more thermal energy is needed to initiate melting in the absence of amylose-rich amorphous regions.

Table (3) :Thermal properties of native rice starch and its derivatives.

Samples	T _o (°C)	T _c (°C)	T _p (°C)	ΔH (J/g)	%R
Native starch	101.79	2.549	-	-	-
Starch acetate	108.68	2.176	276.63	-225.011	-29.2222
Gelatinized starch (a)	100.74	3.227	269.83	43.310	3.4928
(b)	-	-	280.49	-472.871	-38.1348

*Temperatures: T_o onset, T_c conclusion, T_p peak, ΔH enthalpy range and %R retrogradation rate.

The origin of the second peak endothermic transition observed is of particular interest. During gelatinization, double helical and crystalline structures are disrupted in starches. This order–disorder phase transition showed melting of crystals at different temperature ranges in the starch samples, due probably to the difference in starch granular morphology and to the rigidity difference in starch granules in close contact. In addition, the association between amylose and amylopectin molecules in starches, being different from that of individual starch, induced specific chain interactions during gelatinization and each starch gelatinized independently of the other. However, this phenomenon should also be accompanied by a potential competition between the starch granules for water during hydration and swelling. Moreover, starch acetate was characterized by a high transition temperature, which provided structural stability and made its granule more

resistant to gelatinization. starches onset temperature coincided with the later part of the T_c endotherm of the less stable starches and its impact was illustrated by a second peak that corresponded to the more stable peak. The second peak area increases in size when the amylose ratio increase. As observed from the two endotherms, the lowest values of T_p (observed from the first thermogram) corresponded to those of starch acetate and starch phosphate highest values T_p (from the second endotherm) corresponded to those of gelatinized starch with a slight shift to higher values compared to those of individual starches (controls). However, no statistical difference in the T_p of second peaks.

For rice starch samples, the differences in R values are due to a lack of homogeneity that implies a large amount of crystals having different stabilities, or to the presence of crystalline regions of different strength. In addition, the differences in ΔH (reported to reflect the loss of double helix) values in starch mixtures should be attributed to the differences in the amounts of longer chains in amylopectin that require a high thermal energy to disorganize their structure (Yamin *et al.*, 1999), and to a lack of homogeneity of ordered structures inside granules and outside as well.

A general tendency of increasing T_o and T_c when the proportion of the starch of highest onset gelatinization in sample was increased, and a net shift in the values of T_c was also observed in the rice starch phosphate. A comparison between means among the parameters T_o , T_p , T_c and enthalpy of gelatinization showed generally a net significant difference.

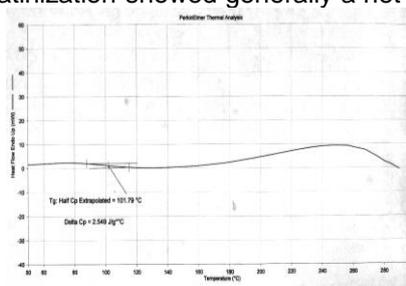


Fig. 7. Thermal properties of native rice starch

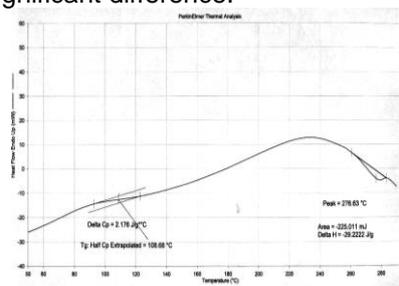


Fig. 8. Thermal properties of starch acetate.

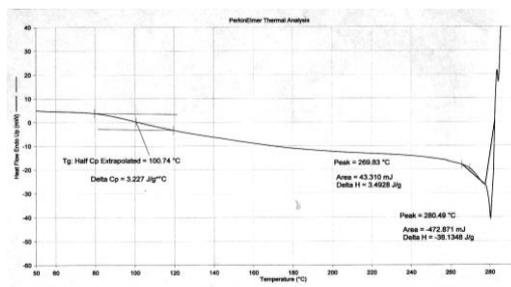


Fig. 9. Thermal properties of gelatinized starch

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دراسات على الصفات الطبيعية لنشا الأرز الطبيعي وسابق الجلتنة وأسيتات النشا.
علاء الدين سلامة محمد* ، جمال رويشد حمد* ، عفت إسماعيل سليم**، على عز العرب
محمد ابوالحسن** ، عبد البصير شرف السيد** و عاطف عبدالله محمد ابوزيد**.
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** قسم علوم وتكنولوجيا الاغذية بالمركز القومي للبحوث - الدقى - الجيزة - مصر.

تم في هذا البحث تحويل نشا الأرز بطريقة طبيعية وهى تكوين النشا سابق الجلتنة وبطريقة كيميائية وهى تحضير أسيتات النشا وبعدها تم دراسة الصفات الطبيعية للنشا المعدل الناتج ومقارنتها بمثيلاتها فى النشا الطبيعي حيث تم تقدير بعض الصفات مثل نسبة الأميلوز ودرجة الذوبان ونسبة الانتفاخ والذوبان على حرارة ٢٥ م° و ٩٥ م°، فحص الشكل المورفولوجى لحبيبات النشا تحت الميكروسكوب الالكترونى والفحص بأشعة X وأخيرا تم دراسة الصفات الحرارية بواسطة جهاز differential scanning calorimeter.

وأوضحت النتائج أن نسبة الأميلوز فى النشا سابق الجلتنة أعلى بكثير منها عن النشا الطبيعي فى حين أن هذه الزيادة كانت بسيطة جدا فى أسيتات النشا.

اما بخصوص نسبة الانتفاخ ودرجة الذوبان ارتفعت فى النشا سابق الجلتنة بدرجة أعلى منها فى أسيتات النشا والنشا الطبيعي.

أوضح الفحص للشكل الظاهري تحت الميكروسكوب الالكترونى أن أسيتات النشا والنشا الطبيعي لم يختلفا كثيرا من حيث الشكل المورفولوجى إلا أن أسيتات النشا كان حجم حبيباتها اقل قليلا من النشا الطبيعي أما فى النشا سابق الجلتنة فقد الشكل البللورى وزاد حجم الحبيبة بدرجة كبيرة عن حجمها فى النشا الطبيعي.

ومن خلال الفحص بأشعة X وجد أن النشا الطبيعي وأسيتات النشا مازالت محتفظة بشكلها البللورى وتكاد العينات أن تكون متماثلة أما النشا سابق الجلتنة فقد جزء كبير من صورته البللورية.

و أوضحت دراسة الصفات الحرارية أن onset temperature (T_o) and peak temperature (T_p) زادت فى أسيتات النشا وانخفضت فى النشا سابق الجلتنة مقارنة بالنشا الطبيعي أما enthalpies of gelatinization ΔH_{gel} زادت فى النشا المحور (اسيتات النشا والنشا السابق الجلتنة) عن مثيلتها فى النشا الطبيعي.